Life Table Parameters, Reproductive Rate, Intrinsic Rate of Increase, and Estimated Cost of Rearing *Podisus maculiventris* (Heteroptera: Pentatomidae) on an Artificial Diet

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ABSTRACT The impact of an insect-free artificial diet provided at nymphal and/or adult stage upon the developmental rate, life table parameters, and fertility table parameters was examined for *Podisus maculiventris* (Say). This study showed that when fed an insect-free artificial diet during both the nymphal and adult stage, developmental time was prolonged, preoviposition period was extended, and reproductive rate (R_0) and intrinsic rate of increase (r) were significantly lower than when fed larval insect prey at both nymphal and adult stages. Additionally, feeding larval prey to adults reared as nymphs on an artificial diet significantly increased the proportion of fertile females, the number of eggs laid by mated females, the reproductive rate and intrinsic rate of increase, but the mean generation time was not significantly different. Likewise, feeding artificial diet to adults reared on larval prey resulted in a significant reduction in reproductive rate and intrinsic rate of increase. The "realized" cost to rear *P. maculiventris* on the artificial diet was calculated (as the cost to double the population size) using raw material cost, fertility table parameters and doubling time values. Raw material cost for rearing *P. maculiventris* colony on *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) was only 1.4 times higher than the cost of artificial diet raw materials required to rear the same size colony. However, the realized cost of rearing was 3.5 times higher when rearing on artificial diet because of the prolonged developmental time and reduced reproductive output. The cost efficiency of rearing a beneficial insect on an artificial diet that decreases the intrinsic rate of increase of a colony is discussed, as well as the advantages and disadvantages of supplementing adult diets with natural prey at the reproductive stage.

KEY WORDS *Podisus maculiventris*, beneficial insect, predator, intrinsic rate of increase, fecundity, rearing

Podisus maculiventris (Say) is a generalist predator native to North America and commonly found feeding upon eggs, larvae and adults of many agricultural and forestry pest insects (Mukerji and LeRoux 1969; Waddill and Shepard 1975; McPherson 1980, 1982; Drummond et al. 1984; De Clercq and Degheele 1992, 1997; Hough-Goldstein and McPherson 1996; De Clercq et al. 1998b; Yeargan 1998). There are several artificial diets available for rearing this beneficial insect; however, rearing *Podisus* on many of the diets has resulted in an increase in developmental time and a decrease in fecundity (De Clercq and Degheele 1992, Greany and Carpenter 1996, De Clercq et al. 1998a, Thompson 1999, Rojas et al. 2000, Wittmeyer et al. 2001). Artificial diets and mass rearing programs for beneficial insects, which have been to date "tedious and expensive" (Thompson 1999), are usually developed to decrease labor and cost of rearing. There is a need, in both the laboratory research environment and in greenhouse, forestry, and agricultural field environments, for a low cost system designed to rear large numbers of multigenerational beneficial insects entirely on artificial diets devoid of insect material (Glenister 1998, Glenister and Hoffmann 1998, Ruberson and Coll 1998, Thompson 1999). In order for mass rearing and augmentative release to become viable as an alternative to pesticide use and other pest control measures, it must prove to be cost effective, with large numbers of beneficial insects available upon demand. Although there is success in the ability to rear successive generations of some parasites and predators entirely on artificial diets, in many cases, there is a significant loss of both fitness and reproductive potential (De Clercq and Degheele 1992; Carpenter and Greany 1998; Thompson 1999; Adams 2000a, 2000b; Rojas et al. 2000).

Although diets are available and have been developed to rear predaceous pentatomids for continuous generations, a substantial prolongation in developmental time and a reduction in fecundity and fertility occurs. This increase in developmental time and decrease in fecundity, may force the need for larger colonies to maintain the numbers needed for augmentative release and, thus, increase the realized cost of

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rearing (the cost to double the population size). Research with other insects has shown that a shift in fecundity, delay of oviposition or an increase in developmental rate to adult significantly decreases the intrinsic rate of increase of an insect population (Chen and Hsiao 1984). The outcome of a lower intrinsic rate of increase, when applied to rearing and augmentative release of beneficial organisms, is a potential increase in cost. The impact of a lower intrinsic rate of increase and its cost in production and rearing must be weighed against the already high cost of rearing beneficial insects on natural prey.

Life tables and fertility tables are powerful tools for analyzing and understanding the impact that an external factor has upon the growth, survival, reproduction, and rate of increase of an insect population (Landahl and Root 1969, Bellows et al. 1992). Fertility tables have been used with many species of insects to improve rearing techniques by evaluating differences in reproductive rate, number of females per female within a generation (Birch 1948) and the intrinsic rate of increase, a constant value used to determine the population increase in an unlimited environment, essentially the difference between birth rate and death rate (Birch 1948) when given different food sources (Fouly et al. 1995, Valicente and O'Neil 1995, Hodek and Honek 1996, Souissi and Le Ru 1997, Richard and Evans 1998, Hansen et al. 1999).

In this study, fertility table parameters were used to examine the impact that a non-natural insect-free food source (i.e., an artificial diet) has upon the developmental rate and rate of increase of *P. maculiventris.* In addition, the intrinsic rate of increase, converted to doubling time, was used to calculate the realized cost of rearing (calculated as the cost to double the population size), to evaluate the cost efficacy of the artificial diet tested.

Wittmeyer et al. (2001) showed that the nutritional quality of food consumed during both nymphal and adult stage of development influenced the fecundity and fertility of adult female *P. maculiventris.* When *P. maculiventris* fed upon an insect-free artificial diet during either nymphal and/or adult stage, their fecundity was reduced significantly. However, it is not known, for this artificial diet, if the decrease in fecundity is large enough to detrimentally affect the reproductive rate and intrinsic rate of increase at a level that would no longer be an improvement in rearing cost. Likewise, Wittmeyer et al. (2001) found that when adult female *P. maculiventris* fed on larval prey, after nymphal development on artificial diet, their reproductive capacity (ovarian maturation, fertility and survivability of eggs) was significantly improved. The current study was designed to evaluate the impact of the artificial diet on the intrinsic rate of increase and to determine if feeding larval prey to adult *P. maculiventris* reared on artificial diet as nymphs improves their reproductive rate and intrinsic rate of increase to decrease cost of rearing.

Materials and Methods

Experimental Design. *P. maculiventris* eggs were obtained from ≈240 females from 24 adult cages of a prey-fed colony maintained on *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuiidae) as defined in Wittmeyer et al. (2001). The eggs were collected on three separate days. Eggs collected each day were defined as a cohort, a group of individuals beginning life together (Birch 1948). Hatching of eggs and growth of first instar nymphs was allowed to occur in half pint paper containers lined with a moist paper towel. Due to the lack of feeding during the first stadium, no developmental data were taken during this stage. When the second-instar nymphs emerged the treatment diets were administered. However, preliminary data from test insects showed that eggs hatched in 5 d, and the nymphs molted to the second instar in 2 d at 26 ± 5°C, 65 ± 10% RH, and a photoperiod of 16:8 (L:D) h. Additionally, the percent hatch and survival to molt from the first instar to the second were on average 86% and 86%, respectively. These values were used in survivorship, generational mortality and fertility table calculations for all treatments.

Newly ecdysed second-instar nymphs were collected within 8–12 h of molting, weighed individually and isolated in half pint paper containers. For each day of collection, a randomly selected sample of 100 isolated nymphs per treatment was set up as a cohort (experimental trial). Each treatment (prey-fed and artificial diet-fed) had a total of three cohorts with 100 isolated individuals in each cohort. From each cohort a random sample (Snedecor and Cochran 1989) of 20 nymphs was taken and weighed individually using an electronic balance (Mettler, AB 204; Toledo, Switzerland) with precision of 0.1 mg. Weights were taken every five days, on days 0, 5, 10, 15, and 20 postemergence of the second instar. Prey-fed nymphal weights were not taken on day 20 because all nymphs had molted to adults before 20 d postemergence of the second instar, while artificial diet-fed nymphs had not yet molted to adults. If a randomly selected individual died during the experimental time, no replacement weight was taken. Daily observations of development and mortality were made for all individuals. Molting was determined by the presence of exuviae and recorded daily. Developmental time of nymphal stages was measured as time (days) within each stadium and time (days) from second stadium to eclosion of adult. Life table values of l_x (number of individuals alive at beginning of stage x) and d_x (number of individuals dying in stage x) were obtained for each stage (x), and used to calculate stage specific mortality and generational mortality within each cohort of nymphs. The starting number of eggs for each cohort ($l_0 = 135$), was computed using preliminary data on hatch and survival to second stadium (with $n = 100$ at start of second stadium).

The artificial diet used in this experiment was a blended buffered mixture of beef liver, whole egg, L-glutamine, and tissue culture media (Ex-Cell 401; JRH Bioscience; Lenexa, KS) encapsulated in a Mylar

(Clear Lam 1992; Jefferson Smurfit, Schaumburg, IL) -Parafilm (52858–032; American National Can, Chicago, IL) dome of 40-μl volume. Preparation and packaging of artificial diet is described in full in Wittmeyer et al. (2001). The natural prey used were late third- and early fourth-instar larvae of *T. ni* coddled at 60°C for 60 s to kill larvae and prevent feeding of isolated nymphs by live *T. ni* larvae. Isolated individual prey-fed nymphs and adults were given two coddled larvae per predator every 24 h. Artificial diet domes were replaced every 48 h; second and third instar received one dome, third and fourth received two domes, and adults received four domes.

Individuals were sexed at adult emergence, and weighed at 3 d postemergence. Additionally, as individuals molted to the adult stage they were randomly selected to be in one of four treatments. The adult treatments were as follows: (1) PnPa, males and females were given larval prey as nymphs and as adults throughout the experimental period; (2) DnDa, males and females were given artificial diet as nymphs and as adults throughout the experimental period; (3) DnPa, males and females were given artificial diet as nymphs and larval prey as adults; (4) PnDa, males and females were given larval prey as nymphs and artificial diet as adults. A total of 59 PnPa females (cohort 1 = 17, cohort 2 = 23, and cohort 3 = 19), 69 DnDa females (19, 26, and 24), 34 DnPa females (11, 9, and 14), and 27 PnDa females (9, 11, and 7) were mated to measure the reproductive parameters for each treatment. The uneven sample sizes were due to mortality of individuals before adult eclosion or mating.

Five days postemergence, PnPa, DnDa, DnPa, and PnDa females were paired with males of the same treatment and allowed to mate. Males were removed after 8 h (to prevent cannibalism of diet-fed adults) and every 48 h each female was given the original male to mate with, unless a male died; then the male was replaced with a virgin male of similar age and rearing regime. Mortality of females was recorded daily and dead females were not replaced. Eggs were collected daily for 12 d after the initiation of mating (up to day 17 postemergence of adult female). Eggs were counted, observed daily for hatch and molting of first-instar nymphs to second instar.

To examine the impact of the artificial diet upon population growth, fertility table parameters were calculated for each cohort and adult treatment (PnPa, DnDa, DnPa, and PnDa). All life table and fertility table parameters were measured and calculated as described in Birch (1948) and Abou-Setta et al. (1986).

Statistical Analysis. All statistics were performed on SAS system software (1989–1996). A split-plot analysis (Gill and Hafs 1971) was performed for the repeated measurements (Rao 1998) of nymphal weights (dependent variable), where the independent variables were treatment (prey-fed and diet-fed), cohort (1, 2, and 3), and day (0, 5, 10, and 15). All original data were tested for normality using an univariate analysis and the *W*-test for normality (Shapiro and Wilk 1965, SAS Instititute 1982). To deal with the non-normality and skewed distribution frequency of developmental time, a rank transformation (Spearman's rank correlation analysis) (Conover and Iman 1981) was performed on all developmental time (times to each stadium and to adult) measurements with treatment (prey-fed and diet-fed) as the independent variable. Rank transformed data for developmental time were evaluated using a general linear model (GLM) to test the effect of treatment with a type III error removing the effect of cohort within treatment, and with means separated by Fisher's Least Significant Difference (LSD) test for significance at $P = 0.05$. Additionally, to examine the effect of sex on developmental time to adult, a GLM was used on rank transformed data [analyzed as a $2 \times 2 \times 3$ (treatment × sex × cohort) factorial with a type III error of cohort within treatments]. Developmental time values were reported as the mean ± SD. Stage-specific mortality and generation mortality cohort by treatment values were analyzed using a randomized complete block analysis of variance (ANOVA) of arcsine transformed values; means were compared by Fisher's LSD test at $P = 0.05$ (SAS Institute 1990), and values were reported as mean ± SD.

For normally distributed data (e.g., female-mated developmental time, preovipositional adult weight, preoviposition period, and eggs per female) a GLM was used on original data to test the effect of treatments using a type III error of cohort within treatment; means were separated by Fisher's LSD test at $P = 0.05$ (SAS Institute 1990), and were reported as least significant means (LSM) ± SE.

Fertility table age specific fecundity, m_x (the number of female eggs laid per female at time x) and adjusted m_x (number of female second-instar nymphs per female at time x) values included all fertile and infertile females to provide a more accurate estimate of reproductive rate (R_0), mean generation time (T) and intrinsic rate of increase (r). Cohort (1, 2, and 3) by treatment (PnPa, DnDa, PnDa, DnPa) values for proportion of fertile females, mortality and all fertility table parameters (T, R_0, r, and adjusted values) were analyzed with a randomized complete block design and ANOVA (treatment means compared by Fisher's LSD test). Proportional data (fertility and mortality) was arcsine transformed before analysis. For all ANOVA tests, significance was evaluated at $P < 0.05$ and values were reported as mean ± SD (SAS Institute 1990).

Analysis of Rearing Cost. Several assumptions were made when calculating the estimated cost of producing *T. ni* larvae and the artificial diet. The overhead facility costs and salaries of workers were not included in the cost of production. Calculations were based on the minimum colony size for maintaining a healthy stable *T. ni* colony; estimated to be six cages of adults with 100 females per cage, and females replenished weekly. The size of the *P. maculiventris* colony was held at 10 cages of 12 adult females cycled out at a weekly basis to ensure the stability of the colony. Production cost was calculated as a cost per day and was based on the cost of raw materials for the *T. ni* colony or artificial diet multiplied by the proportion of

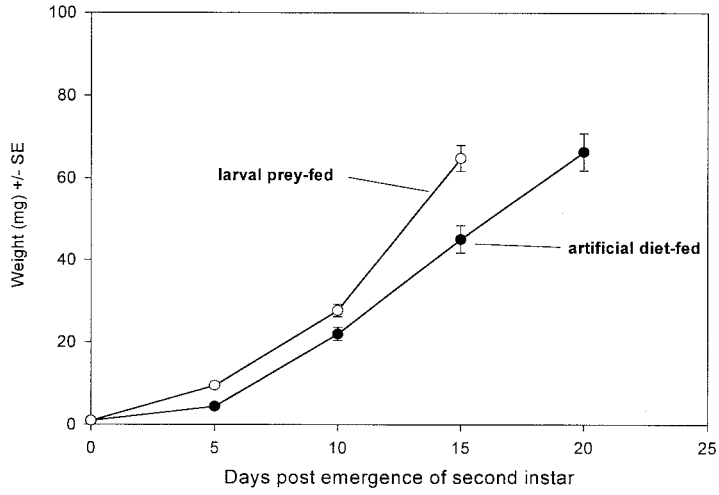

Fig. 1. Nymphal weight of *P. maculiventris* reared on larval prey and an artificial diet. Data points represent treatment least significant means and error bars denote standard error of the means.

hours spent in rearing (based on proportion of a full-time employee's hours) of both the *T. ni* colony and the *P. maculiventris* colony. With the previous assumptions, the estimated cost to maintain a *T. ni* colony was determined to be \$3.21/d and the estimated cost to produce the artificial diet was determined to be \$1.43/d. It was also estimated to cost \$2.57/d to feed *P. maculiventris* colony with either source of food, *T. ni* larvae or artificial diet.

The total cost per generation (TCG) for all treatments was calculated by the following equation:

$$TCG = (n_e)(c_t) + (n_n)(c_f + c_t + c_d) + (n_a)(c_f + c_t + c_d)$$

where n_e = number of days as eggs and first instar (7 d), c_t = cost per day to maintain *T. ni* colony (if prey-fed at any stage, then c_t = \$3.21; when DnDa, then c_t = \$0), n_n = number of days as nymphs from molt of second-instar to adult molt, n_a = number of days as adults (17 d for both prey-fed and diet-fed), c_f = cost to feed *P. maculiventris* when fed either food source (\$2.57), c_d = cost per day to produce artificial diet (dependent upon food source as nymph or adult: when P, then c_d = \$0; when D, then c_d = \$1.43). Both DnPa and PnDa treatments included the cost of maintaining a continuous *T. ni* colony during all stages of development. Hence, PnDa treatment total cost included an additional cost of producing *T. ni* larvae during the egg and adult stage, while DnPa treatment included an additional cost of producing *T. ni* larvae during both egg and nymphal stage.

For all treatments, cost per egg (CPE) was then calculated by the following equation:

$$CPE = (TCG)/[(e)(f)(s)]$$

where e = average number of eggs laid per fertile female; f = number of fertile females surviving to end of egg laying period, and s = proportion of eggs laid that survived molt to second stadium.

To evaluate the effect of intrinsic rate of increase upon the cost of colony maintenance, the number of generations for the population to double (T_d) for PnPa, DnDa, DnPa and PnDa was calculated using mean adjusted r and T values by the equation

$$T_d = [(\ln 2)/\text{adj.r}](1/\text{adj.T}).$$

The "realized" cost (the cost to double the population size) was determined as the total cost per generation times the doubling time (T_d) in units of generation.

Results

Effect of Artificial Diet on Nymphal Weight. A significant treatment effect on nymphal weight was found when analyzed as a split plot analysis for re-

Table 1. Mean developmental time for each stadium and from second stadium to adult of *P. maculiventris* reared on larval prey or an artificial diet

Treatment	2nd instar	3rd instar	4th instar	5th instar	2nd to adult[a]
Prey-fed	3.78 ± 0.84a	4.00 ± 1.48a	4.46 ± 1.68a	6.00 ± 1.34a	18.07 ± 3.73a
	n = 283	*n* = 268	*n* = 261	*n* = 249	*n* = 249
Diet-fed	5.66 ± 1.57b	4.53 ± 1.07a	4.68 ± 1.10b	7.44 ± 1.84b	22.15 ± 2.86b
	n = 282	*n* = 263	*n* = 247	*n* = 223	*n* = 223

Developmental time mean ± SD (days) followed by the same letter are not significantly different at $P < 0.05$ using rank transformation and GLM analysis with a type III error term e = cohort within treatment (SAS Institute 1990), data pooled for sex.

[a] Adult developmental time measured from molt of second stadium to the eclosion of adult (days).

Table 2. Stage specific and generational mortality of *P. maculiventris* fed larval prey or an artificial diet

Treatment	2nd instar[a]	3rd instar[a]	4th instar[a]	5th instar[a]	Generation mortality (egg-adult)[b]
Prey-fed	5.67 ± 3.05a	5.29 ± 2.04a	2.59 ± 2.82a	4.64 ± 2.09a	38.52 ± 3.23a
Diet-fed	6.00 ± 1.73a	6.70 ± 4.49a	6.02 ± 4.41a	9.82 ± 10.31a	44.94 ± 7.27a

Treatment means ± SD for stage specific mortality and generation mortality followed by the same letter were not significantly different with Fisher's LSD test at $P = 0.05$.

[a] Stage specific mortality = $(d_x/1_x)$*100%: with d_x = the number of individuals dying in stage x and 1_x = the number of individuals alive at beginning of stage x.

[b] Generational mortality = $\Sigma(d_x/1_0)$*100%: with dx = the number of individuals dying in stage x and 1_0 = the number of individuals alive at beginning of egg stage; where 1_0 for each cohort was 135 eggs.

peated measurements with type III error of cohort within treatment ($F = 120.3$; df = 11, 425; $P = 0.0001$). The starting weights of nymphs at emergence of second-instar nymphs were the same ($P = 0.9965$) for prey-fed and artificial diet-fed treatments (Fig. 1). However, by day 5 postemergence a significant difference ($P = 0.0001$) between prey-fed and diet-fed weight was found. On day 15 postemergence of the second stadium, mean nymphal weight of prey-fed and diet-fed was 65.09 ± 1.74 mg and 45.19 ± 1.85 mg (LSM ± SE), respectively ($P = 0.0001$). Nymphal weights for prey-fed treatment were not measured for day 20 because all nymphs eclosed to adult before day 20 (Table 1). However, mean nymphal weight for artificial diet-fed nymphs on day 20 was 66.30 ± 4.51 mg (LSM ± SE) (Fig. 1), approximately the same as prey-fed nymphal weight on day 15. Before adult molt, nymphs reared on the artificial diet attained the same weight as the prey-fed controls, but took 5 d longer.

Effect of Artificial Diet on Nymphal Developmental Time. No significant difference between sexes was observed in developmental time ($F = 0.00$; df = 1, 469; $P = 0.9871$). Therefore, data for the sexes was combined in subsequent analyses. Developmental time to adult for nymphs fed artificial diet was extended by an average of 4 d (Table 1). The mean developmental time from second stadium to adult across all prey-fed cohorts (18.07 ± 3.73 d) was significantly shorter than mean diet-fed developmental time (22.15 ± 2.86 d; $F = 453.30$; df = 1, 471; $P = 0.0022$). Significant differences for developmental time within stadia were found in second ($P = 0.0302$), fourth ($P = 0.0213$) and fifth stadia ($P = 0.0047$); whereas, the third stadium showed no significant difference between prey-fed and diet-fed within stage developmental time ($F = 17.25$; df = 5, 530; $P = 0.0534$).

Effect of Artificial Diet on Survivorship, Stage-Specific Mortality Parameters and Generation Mortality. Average percent survival from egg to adult was 61.33% for prey-fed treatment and 54.93% for artificial diet-fed treatment. From egg to third stadium the survival of nymphs remained the same for prey-fed and diet-fed. No significant difference between treatments (prey-fed and diet-fed) was found for stage-specific apparent mortality nor generation mortality (Table 2), because of the high variation between cohorts.

Effect of Artificial Diet on Adult Female Reproductive Parameters, Fertility Table Parameters. Adult mortality of mated females, measured only during the experimental mating period, was varied across all treatments and cohorts (data not shown), with no significant treatment effect ($F = 0.88$, df = 3, $P = 0.5028$). Developmental time to adults for females used to measure reproductive parameters adhered to the same pattern as reported in the previous section on nymphal developmental time (Table 1), with females given prey as nymphs (PnPa and PnDa) reaching adulthood 3.65–6.08 d earlier than females given artificial diet as nymphs (DnDa and DnPa) (Table 3). The mean preoviposition period, female weight, proportion of fertile females and eggs per female during the 12 d observation period for PnPa was significantly

Table 3. Average developmental time, adult weight, preoviposition period, eggs per female, and fertility of female *P. maculiventris* mated for fertility table experiment

Treatment		Dev. time (days)[a]	Weight (mg)[b]	Pre-ovip period (days)[c]	Eggs per female[d]	Fertility[e]
PnPa	$n = 59$	17.84 ± 0.29a	93.68 ± 3.07a	7.27 ± 0.29a	120.09 ± 3.47a	0.8718 ± 0.1125a
DnDa	$n = 69$	21.49 ± 0.27c	69.70 ± 2.83b	9.33 ± 0.32b	24.51 ± 3.22d	0.5297 ± 0.0754b
DnPa	$n = 34$	22.30 ± 0.40c	70.90 ± 4.13b	9.00 ± 0.40b	51.32 ± 4.69c	0.8786 ± 0.0559a
PnDa	$n = 27$	16.22 ± 0.44b	96.79 ± 4.57a	7.09 ± 0.45a	73.16 ± 5.19b	0.8307 ± 0.1500a

Dev. Time, Weight, Preovip. period and Eggs per female values are reported as LSMean ± SE developed from the GLM statistical analysis testing H = treatment, standard errors and probabilities calculated using a type III error term of cohort within treatment (SAS Institute 1990). Fertility values are reported as mean ± SD and significance determined by ANOVA analysis (class trt.: $F = 7.41$, df = 3, $P = 0.0193$). Means within the same column followed by the same letter are not significantly different at $P > 0.05$.

[a] Average developmental time from second stadium to adult for females used in mating study.

[b] Female weight measured on day 3 postemergence of adult, during the preoviposition period.

[c] Number of days from emergence of adult to first oviposition.

[d] Average eggs per female (fertile females only) collected from day 5 to day 17 post-adult emergence.

[e] Number of females that laid fertile eggs (eggs that hatch) per number of total mated pairs.

Table 4. Fertility table parameters for *P. maculiventris* maintained on various feeding treatments of larval prey and an artificial diet

Treatment	Eggs (R_0[a])	Days (T[b])	Rate of increase (r[c])	Adj. R_0[d]	Adj. T[d]	Adj. r[d]
PnPa	42.22 ± 2.95a	34.94 ± 1.96b	0.1109 ± 0.0024a	30.39 ± 2.02a	35.87 ± 1.47b	0.0977 ± 0.0033a
DnDa	7.18 ± 1.26d	39.68 ± 0.95a	0.0497 ± 0.0059d	2.06 ± 0.62c	39.76 ± 1.91a	0.0176 ± 0.0091d
DnPa	15.35 ± 5.54c	40.65 ± 1.52a	0.0667 ± 0.0073c	10.23 ± 4.12b	40.64 ± 1.47a	0.0562 ± 0.0096c
PnDa	22.47 ± 2.39b	31.68 ± 0.89c	0.0995 ± 0.0022b	13.12 ± 1.09b	32.55 ± 0.72c	0.0798 ± 0.0022b

Mean ± SD treatment values within a column followed by a different letter are significantly different with Fisher's LSD test at $P = 0.05$.

[a] $R_0 = \Sigma(l_x m_x)$; number of female eggs per female per generation (1:1 sex ratio is assumed), where l_x = the proportion of mated females alive at time x; and m_x = age specific fecundity or the average daily number of eggs laid from females mated per treatment divided by 2 to compensate for the 1:1 sex ratio of progeny.

[b] $T = \Sigma(xl_x m_x)/R_0$; mean generation time in days.

[c] $r = \Sigma e^{-rx} l_x m_x$; intrinsic rate of increase.

[d] Adjusted values take in account the mortality of eggs and first-instar nymphs.

higher than for DnDa (Table 3). Feeding larval prey to *P. maculiventris* at the adult stage after nymphs were fed artificial diet (DnPa) did not significantly improve preovipositional weight, nor preovipositional period compared with DnDa values (Table 3). However, for the DnPa treatment the average eggs per female and the proportion of fertile females was significantly higher than DnDa treatment (Table 3). Feeding artificial diet during the adult stage only (PnDa) significantly reduced average eggs per female compared with PnPa treatment, but showed no significant change in female weight, preovipositional period or fertility (Table 3). The nymphal feeding regimen showed significant influence on the weight, preovipositional period, and eggs per female. However, any feeding regimen that included larval prey (PnPa, DnPa, and PnDa) had significantly higher fertility than that which provided only artificial diet (DnDa) (Table 3).

The mean reproductive rates (R_0) and intrinsic rates of increase (r) for all treatments were significantly different from each other, with females fed larval prey during the nymphal stage (PnPa and PnDa) having the highest rates out of the four feeding regimens (Table 4). Feeding prey to adults reared on artificial diet through the nymphal stage (DnPa) significantly increased the R_0 to more than twice the rate of adults continually fed artificial diet (DnDa). However, in the DnPa treatment, because the mean generation time (T) was significantly longer and the reproductive rate (R_0) significantly lower than PnPa, the intrinsic rate of increase (r) improved by only 0.017 compared with that in DnDa and was still significantly lower than the intrinsic rate of increase (r) for PnPa. Additionally, feeding artificial diet to adults after rearing on larval prey through the nymphal stage (PnDa) decreased R_0 to almost half the reproductive rate of adults continually fed larval prey (PnPa). Despite the low mean generation time (T) for PnDa, the intrinsic rate of increase (r) was still significantly lower than for PnPa, although nearly twice the rate of that for DnDa. Adjusted values for R_0, T, and r show similar trends, although in the DnDa treatment the R_0 and r values decreased more dramatically (Table 4).

Effect of Artificial Diet on Doubling Time and Cost of Rearing. Doubling time in generations (T_d) was much shorter for PnPa than for all other feeding treatments (Table 5). Doubling time for DnDa was five times longer than for PnPa treatment. Additionally, adults fed larval prey (DnPa) showed improvements in population growth, reducing the doubling time to 0.687 generations less than the DnDa treatment doubling time. Feeding the artificial diet only at the adult stage also increased the doubling time of PnDa treatment to 1.35 times longer than PnPa (Table 5).

The cost of raw materials (total cost/generation) required to feed one generation was higher for all treatments fed larval prey at any stage (PnPa, PnDa, and DnPa), as compared with the cost of materials for rearing one generation on artificial diet continously (DnDa) (Table 5). However, due to the higher number of eggs laid, shorter mean generation time and

Table 5. Doubling time and cost of rearing for *P. maculiventris* reared on larval prey and an artificial diet

Treatment	Doubling time (T_d)[a]	Total cost per generation[b]	Cost per egg[c]	Cost of doubling[d]
PnPa	0.197	$225.41	$0.1605	$ 44.40
DnDa	0.9904	$157.08	$3.3329	$155.57
DnPa	0.3034	$281.51	$0.3786	$ 85.41
PnDa	0.2668	$249.71	$0.2378	$ 66.63

[a] Doubling time, T_d (generations) = $[(\ln 2)/r](l/T)$; calculated using mean adjusted r (intrinsic rate of increase) values and mean adjusted T(days/generation) values for each treatment.

[b] Total cost per generation (TCG) = $(n_e)(c_t) + (n_n)(c_f+c_t+c_d) + (n_a)(c_f+c_t+c_d)$; where n_e = number of days as eggs and first instar, c_t = cost per day to maintain *T.ni* colony, n_n = number of days as nymphs from molt of second instar to adult molt, n_a = number of days as adults, c_f = cost to feed *P. maculiventris* when fed either food source, and c_d = cost per day to produce artificial diet.

[c] Cost per egg = $(TCG)/[(e)(f)(s)]$; where e =eggs/ fertile female, f = number of fertile females surviving to end of egg laying period, and s= proportion of eggs laid that survived to molt of 2nd stadium.

[d] Cost of doubling = $(TCG)(T_d)$; realized cost of rearing integrates the intrinsic rate of increase with cost to determine the role of population growth in cost analysis.

faster doubling time for treatments fed larval prey at any stage (PnPa, DnPa, and PnDa), the cost per egg and the cost to double population size (the realized cost of rearing), was lower than for DnDa treatments. Cost per egg for DnDa treatment was >8 times the cost for DnPa treatment and >20 times the cost for PnPa treatment. Likewise, the cost of doubling, or the realized cost of rearing, on artificial diet during both nymphal and adult stages (DnDa) was 3.5 times the cost to rear on larval prey (PnPa). Improved reproduction during the adult stage by feeding larval prey to adults fed artificial diet during nymphal stage (DnPa) reduced the cost per egg by 88.6% and the cost of doubling by almost 45% of DnDa cost, but was still nearly 2.5 times the cost per egg and 1.95 times the cost to double a continuously prey-fed colony (PnPa). In contrast, rearing nymphs on larval prey and then providing artificial diet only during the adult stage (PnDa) did not cause a dramatic increase in cost and was only slightly higher (1.5 times cost per egg and doubling cost) than prey-fed (PnPa) treatment (Table 5).

Discussion

Overall, generational survivorship was not significantly different between prey-fed and diet-fed insects. Additionally, no significant difference was found between prey-fed and diet-fed stage-specific mortality or generational mortality. Female mortality was also not significantly affected by the artificial diet. Weight gain in nymphs on the artificial diet was slower than that in prey-fed nymphs; however, by late fifth instar (day 20 for diet-fed and day 15 for prey-fed) little difference in nymphal weight was seen.

The developmental time to adult of *P. maculiventris* on this artificial diet was extensively prolonged, as was the case with other insect-free artificial diets tested on *P. maculiventris* (De Clercq and Degheele 1992, De Clercq et al. 1998a). However, when fed this artificial diet prolongation of the developmental time of *P. maculiventris* was most pronounced during the second and fifth stadia, in contrast to the diet tested by De Clercq and Degheele (1992) where development was prolonged across all nymphal stadia. This information provides direction for future research on improving artificial diets to reduce developmental time of predaceous pentatomids with the examination of the reasons for sensitivity of second- and fifth-instar nymphs to this artificial diet. Nymphal developmental time for fifth instar may be a good indicator of suitability of the diet when reared from second stadium to adult on the test diet. In contrast, the third and fourth stadia showed little or no significant difference in developmental time and thus would not be the best measures for selection of fit individuals or for testing the effectiveness of improved diets. Additionally, the presence of a high percentage of outliers or extremely slow developers (data not shown) on the artificial diet may reflect a propensity for some individuals to adapt more quickly to the diet. Were that the case, fast developers could be selected to construct a strain more suited to the artificial diet.

Thompson (1999), in a review of the progress in rearing and augmentation of beneficial parasitoids and predators, concluded that to evaluate the true efficacy of an artificial diet for use in augmentative mass rearing, the realized cost of rearing must be considered. In this study the estimated cost of raw materials and labor to produce the artificial diet was low compared with the cost of raising a Lepidoptera colony (*Trichoplusia ni*); however, due to the prolonged development and reduction in eggs per female (hence the decrease in intrinsic rate of increase) the realized cost of rearing on this artificial diet (calculated using doubling time) was more than three times the cost of rearing on the natural prey. Even when larval prey was provided at the adult stage, the realized cost of rearing was two times greater than for predators fed prey continuously. In light of the estimated realized cost, feeding natural prey at the adult stage does not substantially improve the loss in egg production per female sufficient to improve cost-effectiveness. Furthermore, a colony of the natural prey would have to be maintained (at the minimum level for colony health), typically requiring separate facilities and workers from those maintaining the predator colony and producing the artificial diet, cost values which were not included in the estimated realized cost calculations.

Both the reproductive rates and mean generation times were negatively impacted by the artificial diet, leading to a lower intrinsic rate of increase and longer doubling time. Additionally, the intrinsic rate of increase and the doubling time were calculated under isolated conditions; however, in mass rearing environments *P. maculiventris* is highly cannibalistic, especially when fed a sub-optimal diet source (De Clercq and Degheele 1992, Hough-Goldstein 1998, Schmidt et al. 1998). The cannibalism of *P. maculiventris* would very likely add additional increases in the cost of rearing on an artificial diet.

The analysis of both the cost per egg and the cost of doubling based on life and fertility table parameters presented in this study revealed a significant increase in the cost to rear *P. maculiventris* on this artificial diet. In light of this study, the evaluation of artificial diets should be carefully reported and may benefit from a comparison of cost. Furthermore, supplementing the artificial diet-fed treatment with natural larval prey at the adult stage, while improving fertility, mortality and fecundity, did not greatly improve the intrinsic rate of increase or reduce the overall cost of colony maintenance.

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